Evaluation of Biological Compounds of Streptomyces Species for Control of some Fungal Diseases

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Abstract: Fifty cases in cattle farm at Giza governorate were investigated. Some animals were suffered from clinical manifestations such as growth retardation, refused feeds, diarrhea, skin patches, cough and nasal discharge. Hundred samples of air, water supply and feeds including tibn, hay and processed feeds (20 of each) and Sixty samples of feces (of diarrheic animals) skin and nasal swabs (20 of each) were collected for fungal examination. The results revealed that 9 genera of moulds and 2 genera of yeasts were recovered from feed samples. The most predominant isolates of all types of feeds were the mould of genus *Aspergillus* particularly A. *flavus* (95%)**.** Also, members of genus *Aspergillus* were predominantly recovered from most of samples of discharges as it were recovered (47.0 %). On the other hand, only one species of moulds was isolated from the skin scraping associated with skin lesion (*Trichophyton sp.*). Most of isolated *A. flavus* and *A.ochraceus* from animal feeds in diseased farms produced significant levels of aflatoxins and ochratoxins, respectively. The isolated *A. flavus and A.ochraceus* from tibn yielded a higher mean levels of aflatoxins and ochratoxins (2700±3.7 and 3250±2.5ppb), respectively. The antifungal effects of stationary or the exponential culture filtrate obtained from the strain of *Streptomyes sp.* were evaluated against the isolated pathogenic fungi. The results indicated that the stationary culture filtrate possessed a higher antifungal potential than the exponential culture filtrate. Where, the filtrate of the stationary phase of *Streptomyes sp.* yielded significantly wider range of antifungal activity zones ranged from 7±0.69 to 11±1.41 mm diameter compared with antifungal activity zone of the culture filtrate of the exponential phase which ranged from 5±0.64 to 8±1.58 mm diameter in comparison with benzoic acid as control which ranged from 3±0.55to 8±0.83 mm diameter ($P < 0.05$). The production of chitinase (6.0 u/mg protein) and β-1, 3-glucanase (0.82- 0.35 u/mg protein) enzymes by *Streptomyces* were related to fungal growth inhibition and the biological control of fungal pathogens was possible because of the ability of *Streptomyces* to degrade fungal cell walls. MIC_{50-90} of tested antimycotic drugs (Nystatin, Ketoconazole and Itraconazole) as will as *Streptomyces* extract were ranged from 0.75±0.05 to 4±0.81 µg/ml against isolated yeasts (*Candida albicans* and *Rhodotorulla* sp.). *Streptomyces* exponential and stationary culture filtrate as will as its extract could be used as antifungal agent.

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1. Introduction:

Fungal pathogens pose serious problems worldwide for both human and animal health, especially in the subtropical and tropical regions. Fungi and their toxin are natural contaminants of foods and feeds even when the best condition of culture, harvest, storage and handling were used. However the chemotherapeutic agents such as fungicidal drugs are causing residue hazards and deliberating action for human. Hence, up to date the active search for new pharmacologically active agents of natural sources was led to the discovery of many useful drugs which could play important role in treatment of many fungal and mycotoxin diseases with neither environmental pollution nor development of fungicide resistance pathogens. Serious researches are needed to identify alternative methods for human and animal protection, which are less dependent on chemicals. Microbial antagonists are widely used for the biocontrol of fungal diseases. Such microorganisms include bacteria, algae and fungi, of such as bacterium of *Streptomyces spp* (Brimner and Boland, 2003). Many species of actinomycetes, particularly those belonging to the genus *Streptomyces,* are well known as antifungal biocontrol agents that inhibit several pathogenic fungi El-Tarabily *et al.*(2000); Xiao *et al.* (2002) ; Joo, (2005) and Errakhi *et al.*(2007). The antagonistic activity of *Streptomyces* to fungal pathogens is usually related to the production of antifungal compounds (Trejo-Estrada *et al.* (1998); Ouhdouch *et al.*(2001); Getha and Vikineswary.(2002); Fguira *et al.*(2005) and Taechowisan *et al.*(2005) and extracellular hydrolytic enzymes (Valois *et al.*

(1996) ;Mahadevan and Crawford (1997) ; Trejo-Estrada *et al.* (1998) and Mukherje and Sen (2006).

Chitinase and β-1,3-glucanase are considered to be important hydrolytic enzymes in the lysis of fungal cell walls, as for example, cell walls of *Fusarium oxysporum, Sclerotinia minor*, and *S. rolfsii* **(**Singh et al. (1999); El-Tarabily *et al. (*2000) and El-Katatny *et al. (2001)*.The antifungal potential of extracellular metabolites from *Streptomyces* against some fungi was previously reported (Chamberlain and Crawford (1999); Joo,2005 and Fguira *et al.* 2005)**.**However, data related to the antagonistic ability of the extracellular metabolites of *Streptomyces* strains to suppress the growth of the fungal pathogens *C. gloeosporioides* and *S. rolfsii* having a broad host range are limited (Errakhi *et al.* (2007). Therefore the aim of the present work was to screen the environment to fungal contamination and tested for its toxigenicity. Furthermore, evaluation of *Streptomyces spp*s extracts as a biofungicidal compound .

2. Material and Methods

Samples:

One hundred samples of air, water supply and feeds including tibn, hay and processed feeds (20 of each) and Sixty samples of feces (of diarrheic animals) skin and nasal swabs (20 of each) were collected from cattle farm at Giza governorate. Some animals were suffered from some clinical manifestation as diarrhea, cough, nasal discharge, growth retardation, refused feeds.

Standards of aflatoxins and ochratoxin A:

Standards of aflatoxins B1, B2, G1 and G2 and ochratoxin A were purchased from Sigma Chemical Company (USA).

Standards of antifungal agents:

Standards of Benzoic acid, Nystatin, Ketoconazole and Itraconazole were purchased from Sigma Chemical Company (USA).

Isolation and identification of moulds:

The samples were subjected for isolation of fungi as recommended by **(**Refai, 1979) and it were classified according to the keys of Refai (1988) and Conner *et al.* (1992).

Productions of aflatoxin by *A.flavus A.* (Gabal et al., 1994) and *ochraceus* ochratoxin A (Davis et al., 1966) by using liquid medium:

The produced aflatoxins and ochratoxins were extracted and estimated by fluorometric method as described by, Hansen, 1993.

Microorganisms and culture conditions:

1-The fungal strains of fungi which predominantly isolates from the present samples were maintained on Sabouraud,s dextrose agar (oxoid) and grown in Sabouraud,s dextrose broth as described by (Cruikshank *et al.* 1975)

2-The bacterium: The used bacterial strains in this study was *Streptomyces* sp. Which recovered from soil and pure culture of the strain, *Streptomyces* sp. ANU 6277 was maintained on a yeast extract- malt extract- dextrose agar (ISP-2) medium. It was kept in 20% glycerol at –80°C (Cruikshank *et al.* 1975).

Screening for the antagonistic activity of *Streptomyces sp.* against isolated fungi :

The strains of *Streptomyces sp.* were screened for their *in vitro* antagonism against the recovered fungi from cases of animal diseases according to the modified method of Crawford *et al.* (1993), Briefly, a 20-µl suspension of spores (10^6 spores/ml) of *Streptomyces* sp strain was spotted on one side of a potato dextrose agar (PDA) plate and incubated at 28 °C for 3 days. A mycelial plug of 6.0-mm diameter from 3-days-old of each fungus was cut and transferred to an *Streptomyces* sp pregrown PDA plate. The fungal plug was additionally placed on uninoculated PDA plates separately as control treatment. The radial fungal growth in the direction of the antagonist in both the control and the dual culture plates was measured at 4 to 6 days after incubation. The levels of inhibition were calculated by using the equation as previously mentioned by Yuan and Crawford (1995).

Measurement of extracellular chitinase and β-1, 3-glucanase in culture filtrate of *Streptomyces sp.:*

The strain of *Streptomyces* sp was grown in nutrient broth with continuous shaking at 150 rpm at 28 °C for 9 days. Cell-free supernatants were collected at 1-day intervals by centrifugation at 8,000 rpm for 20 min at 4 °C. Cell pellets were dried at 80 °C and weighed to determine the growth. Chitinase activity was quantitatively determined by by the procedure described by Wang *et al.* (2002). The β-1, 3-glucanase activity was measured according to the method of Singh *et al.* (1999), using laminarin from *Laminaria digitata* (Sigma, USA) as substrate. The amount of glucose released by the action of β-1,3-glucanase enzyme was measured by using dinitrosalicylic acid solution (Miller, 1959). Total protein concentration was assayed by the method of using the Bio-Rad protein assay dry reagent (Bio-Rad, USA), with bovine serum albumin Bradford (1976).

In vitro antifungal activity of extracellular metabolites in cell-free culture filtrates of *Streptomyces sp.* (Nakashima *e. al.*, 2002):

To prepare the cell-free culture filtrate *of Streptomyces* sp., the antagonist was cultured into SD broth and incubated on an incubator shaker (150 rpm) at 28 °C. The fermentation broth was collected during the exponential and stationary phases. Cells were removed by centrifugation at 8,000 rpm for 20 min at 4 °C. Cell-free supernatant was filtered aseptically through a sterile membrane with 0.45-µm pore size and stored at 4 °C. The growth inhibitory effects of the extracellular metabolites from culture filtrates were estimated by using the radial growth inhibition assay as described previously by Prapagdee *et al.* (2007) with some modifications. Fungal growth inhibition was expressed as the percentage of radial growth inhibition in comparison with the control (Benzoic acid as 100µg/ml)

Extraction of secondary metabolites

The fermentation process was carried out for six days at 30°C using liquid starch nitrate as production medium, filtered and centrifugated at 5000 r.p.m at pH 7.0 for 20 minutes (Guangying *et al.,* 2005). The clear filtrates containing the active metabolites was collected, and evaporated under reduced pressure using rotary evaporator at a temperature not exceeding 50°C.The active metabolites was precipitated by petroleum ether and centrifuged at 5000 rpm for 15 minute. Its color was pale yellow.

Table

Purification by then layer Chromatography:

The purification of active metabolites was carried out using silica gel column (2.5 X 50) chromatography. Chloroform and Methanol 95:5 (v/v) (Guangying *et al.,* 2005) was used as an eluting solvent. The column was left overnight until the silica gel was completely settled. One-ml crude extract to be fractionated was added on the silica gel column surface and the extract was adsorbed on top of silica gel. Fractions were collected and tested for their antifungal activities. Only one band at R_f = 0.60 showed antifungal activity , identified according to the recommended international references of Umezawa (1977) by ultraviolet (UV) absorption spectrum at 225-321 nm then used for determination of MIC.

Biological activity of the antifungal agent:

The minimum inhibitory concentration (MIC) has been determined by the cup method assay (Kavanagh, 1972).

Statistical analysis

The results (Mean ± S.E) were calculated were analyzed by one-way analysis of variance (ANOVA) as explained by Petrie and Watson (1999). Significant differences at ($p < 0.05$) using SPSS 15

3. Results and Discussion

Table (1): Prevalence of fungi at investigated air, water and feeds (n=20 for each type of samples).

Isolates	Air		Water		Feeds						
					Tibn		Hay		Processed		Total
	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	%
Aspergillus spp.	5	25	8	40	20	100	20	100	17	85	95
A.flavus	3	15	2	10	15	75	20	100	17	85	86.6
A.parasiticus	4	20	1	5	10	50	2	10	10	50	36.6
A.niger	3	15	8	40	8	40	10	50	5	25	38.3
A.ochraceus	0	0	3	15	5	25	5	25	6	30	26.6
A.fumigatus	1	5	2	10	-	-	-	-	1	5	1.6
A.candidus	3	15	1	5	5	25	2	10	1	5	13.3
Penicillium spp.	1	5	1	5	11	55	11	55	14	70	60
Fusarium spp.	1	5	0	0	5	25	12	60	–	–	28.3
Mucor spp.	2	10	0	0	–	–	2	10	10	50	20
Rhizopus spp.	1	5	2	10	1	5	1	5	4	20	10
Candida albicans	2	10	6	30	-	-	–	-	5	25	8.3
Rhodotorula spp.	2	10	4	20	2	10	–	-	8	40	16.6
Cladosporium spp.	4	20	1	5	2	10	10	50	4	20	26.6
Alternaria spp.	2	10	0	0	8	40	10	70	2	10	40
Curvularia spp.	1	5	0	0	–	–	1	5	2	10	5
Scopulariopsis spp.	1	5	0	0	–	–	–	–	5	25	1.6

Table (2): Prevalence of fungi in some body excretions from diseased animals (n=20 for each type of samples).

Fungal Isolates	Feces		Skin scraping		Nasal Swabs		Total
	+ve	%	+ve	%	+ve	%	%
Aspergillus spp.	16	80	0	0	18	90	56.6
A.flavus	14	70	0	0	15	75	48.3
A.parasiticus	10	50	0	0	10	50	33.3
A.niger	8	40	0	0	12	60	33.3
A.ochraceus	10	50	0	0	9	45	31.6
A.fumigatus	2	10	0	0	18	90	33.3
A.candidus	1	5	0	0	6	30	11.6
Penicillium spp.	7	35	0	0	7	35	23.3
Fusarium spp.	1	5	0	0	1	5	3.3
Mucor spp.	1	5	0	0	1	5	3.3
Rhizopus spp.	0	0	0	0	0	0	0
Candida albicans	9	45	0	0	3	15	20
Rhodotorula spp.	2	10	0	0	1	5	5
Cladosporium spp.	2	10	0	0	1	5	5
Alternaria spp.	0	0	0	0	0	0	0
Curvularia spp.	0	0	0	0	1	5	1.6
Scopulariopsis spp.	0	0	0	0	1	5	1.6
Trichophyton T. verrucosum	0	0	8	40	0	0	13.3
T. mentagrophytes	0	0	6	30	0	0	10

Table(3) Screening of A. *flavus* isolated from feeds for production of aflatoxin B_1 (n=20 for each type of samples).

Source of isolate	Incidence		Toxigenicity			Aflatoxin B1 (ppb)		
	+ve	%	+ve	–ve	%	Max.	Min	Mean
Hay	15	75	10	5	33.3	5000	50	1700±5.2
Tibn	20	100	11	4	73.3	6300	35	2700±3.7
Processed feeds	17	100	7	10	41	2000	310	516±1.5

Table(4) Screening of *A.ochraceus* isolated from feeds for production of Ochratoxin (n=20 for each type of samples)

Source of isolate	Incidence		Toxigenicity			Ochratoxin (ppb)		
	+ve	%	+ve	–ve	%	Max.	Min.	Mean±S.E
Hay	5	25	3	2	60	7000	5000	583±0.2
Tibn	5	25	2	3	40	6300	200	3250±2.5
Processed feeds	6	30	3	3	50	3100	470	1400±1.5

Table(5): Evaluation of the antifungal effect of bioactive compounds of streptomyces compared with Benzoic acid(100 µg/ml) by using agar plate diffusion assay as inhibition zone diameter mm (n= 5).

Tested fungi	Bioactive compounds of streptomyces sp		Benzoic acid
	Stationary culture filtrate	Exponential culture filtrate /	
A.flavus	11±1.30^{A}	5±0.73^{a}	4±0.71^{a}
A.parasiticus	10±1.00^{A}	8±0.68^{B}	5±0.69^{ab}
A.fumigatus	7±0.70	5±0.72	5±0.84
A.ochraceus	9±1.13^{A}	8±1.58^{B}	3±0.55^{ab}
Penicillium spp.	7±0.69	5±0.0.71^{A}	8±0.85^{a}
Fusarium spp.	8±0.71^{A}	5±0.73^{a}	5±1.05^{a}
C. albicans	11±1.41^{A}	7±1.14^{a}	6±1.22^{a}
T.mentagrophytees	7±0.73	5±0.69	5±0.83
T. verrucosum	8±0.71^{A}	7±0.84^{B}	4±0.68^{ab}

Small letters a and b in the same raw represent a significant change against capital letters A and B, respectively by LSD (ANOVA) at P= 0.05

Table (6): MIC of *C. albicans* yeasts isolated from clinical cases against tested antifungal compounds (n=15).

Tested compounds	MIC rang (µ/ml)							
	0.125	0.25	0.5	0.75	1.0	2	3	4
Nystatin	R	1±0.15	5±0.43A	8±0.71A	10±1.14A	12±1.30A	13±0.45A	15±0.25
ketoconazole	R	1±0.46	3±0.45a	6±0.89	9±0.71	11±0.71B	12±0.71B	15±0.52
Itraconazole	R	1±0.52	3±0.44a	4±0.71a	7±0.32a	8±0.70ab	10±0.71ab	15±0.44
Streptomyces Extract	R	R	4±0.46	5±0.70a	8±0.72	9±0.72a	11±0.72a	15±0.63

Small letters a and b in the same raw represent a significant change against capital letters A and B respectively by LSD at P= 0.05 (ANOVA), R = resistant

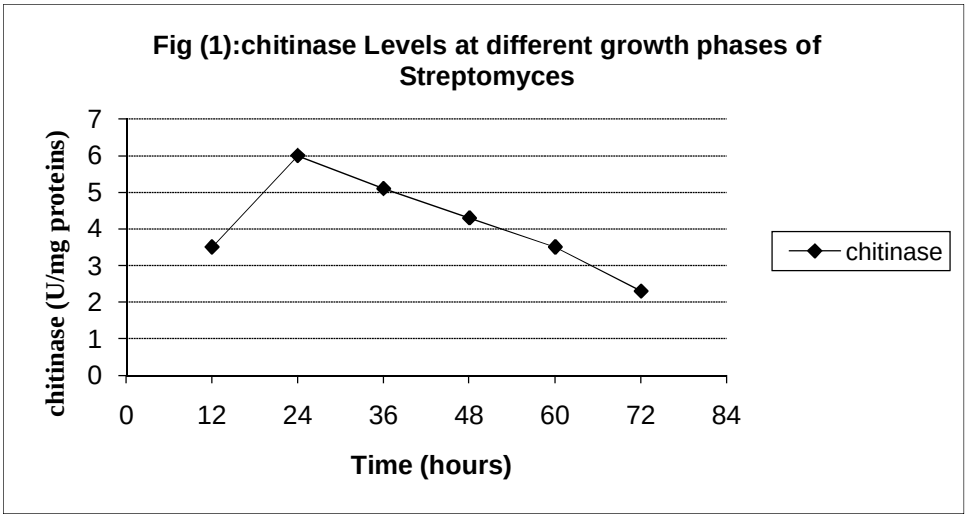
Table (7): MIC range of *Rhodotorula* sp isolates from clinical cases against tested extract and some antifungals. (n=15).

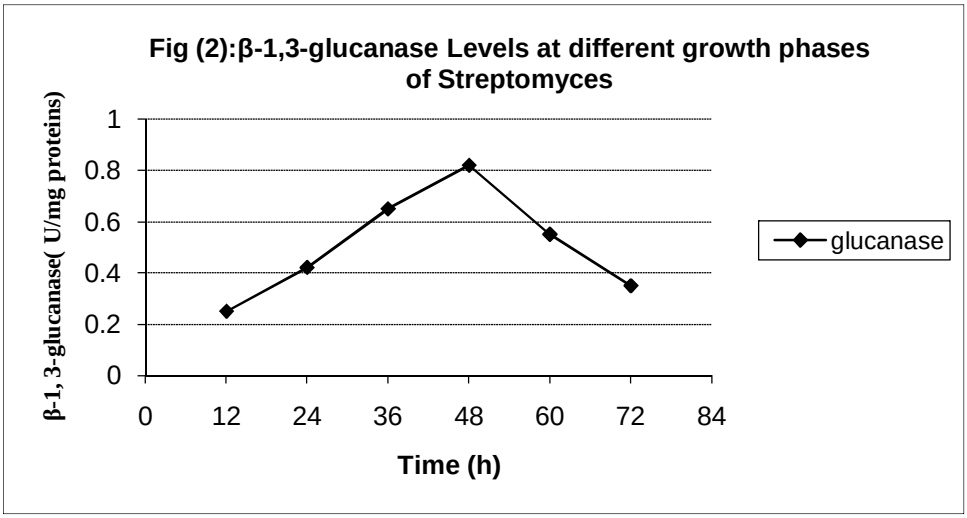
Tested compounds	MIC rang (µ/ml)							
	0.125	0.25	0.5	0.75	1.0	2	3	4
Nystatin	R	2±0.32	3±0.45	7±0.87A	8±0.71A	12±1.3A	14±0.32	15±0.55
ketoconazole	R	R	2±0.32	5±0.71	9±0.71B	11±0.71	13±0.55A	15±0.63
Itraconazole	R	R	3±0.45	4±0.71a	7±0.32b	9±0.71a	15±0.63a	15±0.55
Streptomyces Extract	R	R	2±0.32	5±0.71	6±0.71ab	10±0.89	14±0.55	15±0.63

Small letters a and b in the same column represent a significant change against capital letters A and B, respectively by LSD at P= 0.05 (ANOVA), R = resistant

Table (8): MIC_{50-90} of yeasts isolated from clinical cases against tested extract and some antifungals (µg/ml , n=15).

Fungal isolates	Nystatin		Ketoconazole		Itraconazole		Strept.Extract	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
C. albicans	0.75±0.05	4 ±0.81	1 ±0.05	3 ±0.12	2 ±0.08	4 ±0.25	2±0.16	4±0.46
***Rhodotorulla* sp.**	1 ±0.11	3 ±0.75	1 ±0.07	4 ±0.23	2 ±0.24	3 ±0.14	2±0.27	4±0.28





Mycosis and mycotoxicosis were progressively increased with the intensive environmental pollutions. In the present work, cattles were suffered from clinical manifestation as refused feeding, growth retardation, diarrhea, skin patches, cough and nasal discharge. One hundred samples of air, water supply and feeds including tibn, hay and processed feeds (20 of each) and Sixty samples of feces (of diarrheic animals) skin and nasal swabs (20 of each) were collected for fungal examination. Nine genera of mould and 2 genera of yeast were recovered from feed samples. The most predominant isolates of all types of feeds were the mould of genus *Aspergillus* particularly *A. flavus* (95%) (Table, 1).These results also reported previously by (El-Ghareeb and Khader , 2000 and Hassan *et a*l., 2009)

On the other hand, other genera as Penicillium sp. (60%), Fusarium sp. (28.3%) and Alternaria (40%) of mould were isolated in variable frequency. Whereas, the mould of Curvularia sp. were rarely isolated from feeds (5%). Also, members of genus *Aspergillus* were predominantly recovered from most of samples of 25% of air and 40% of water supply while, in animals discharges as it was recovered from 56.6 % of samples. It was recovered from 80% of faecal samples of cattle diarrhea and 90 % of nasal discharge of cattle suffered from pneumonia (Table, 2).

It was interesting to report that the yeast of *Candida albicans* was isolated in a significant frequency in all samples associated with diseases in cattle, It was isolated from 10% of air, 30% of water, 45% of feces (of diarrheic cases), 15% of nasal

discharge of pneumonic animal. Similar findings were previously reported by (Hassan *et al.,* 2007). Other genera of mould and yeast were also isolated in different frequencies. The *Penicillium species* and *Rhodotorula species* were the most significant fungi after the species of the *Aspergillus* and *C. albicans*. Only, one species of moulds was isolated from the skin scraping associated with skin lesion (*Trichophyton sp.*).These findings supported by the findings of (El-Refai *et al.,* 1977 and Hassan *et al.,* 2007) who reported that the isolation of *Trichophyton* from ring worm cases indicated that skin specially the rough keratin layer is the predilection site of these fungi. Whereas, other fungi could not be recovered from ring worm cases. This may be due to their inability to grow on rough layer of the skin of relatively higher temperature (up to 37°C).

Feeds beside other environmental factors suspected to play important role in animal infections. The dangers of mould and yeast besides causing animal mycosis, they produced fungal metabolites such as mycotoxins, such mycotoxins produced under adverse effect of environmental conditions. These mycotoxins residues in food and feed causes carcinogenic, teratogenic, haemorrhagic and immunosuppression effect to human and animal health (Sayed El Ahl *et al.,* 2006 and Hassan *et al.,* 2007).

Most of isolated *A.flavus* and *A.ochraceus* from animal feeds in diseased farm produced significant levels of aflatoxins and ochratoxins, respectively. The isolated *A.flavus and A.ochraceus* from tibn yielded a higher mean levels of aflatoxins and ochratoxins (2700±3.7 and 3250±2.5ppb ,respectively) as seen in Table,3 & 4 .The detection of mycotoxin in feeds and environmental factors in assosciation with other different animal diseases were also reported by Hassan *et al.* (2007).

The biological approaches to antifungal and mycotxins detoxification will be taken as a mean of bio-transformation or degradation of toxin by endogenous enzyme to a metabolites that is either nontoxic when ingested by animal or less toxic than that the original toxin and readily extracted from the body .In the present study, the antifungal effects of stationary or the exponential culture filtrate obtained from the strain of *Streptomyces sp.* were evaluated against the pathogenic fungi that isolated from diseased cases . The results indicated that the stationary culture filtrate possessed a significantly higher antifungal potential than the exponential culture filtrate and benzoic acid (as control of antifungal activity). Where, the filtrate of the stationary phase of *Streptomyces sp.* yielded a wide range of antifungal activity zones ranged from (7±0.69-11±1.30 mm) in diameter. On the other hand, the antifungal activity zone (Table, 5) of the culture filterat of the exponential phase not exceeded 5±0.69-8±1.58 mm in diameter in comparison with benzoic acid (3±0.55-8±0.85 mm). These findings imply that the antifungal potential of the exponential culture filtrate was probably related to the increased production of hydrolytic enzymes, particularly chitinase. It has been reported that chitinase from *Streptomyes sp.* was able to lysis the cell walls of *fungus* (El-Katatny *et al.,* 2001).There is a possibility that the increased antifungal activity against the fungi tested in these experiments by the stationary culture filtrate of *Streptomyes sp.* is a consequence of the production of extracellular secondary antifungal compounds. The production of secondary antifungal compounds has been already reported in many species of *Streptomyces* (Fguira *et al.,* 2005 and Taechowisan *et al.,* 2005). Antifungal production by *S. hygroscopicus* can inhibit a broad range of fungal pathogens such as *Rhizoctonia solani, Pythium ultimum, F. oxysporum,* and *Sclerotinia homeocarpa* (Chamberlain and Crawford, 1999 a&b*).* In general both exponential and stationary culture filtrate of *Streptomyes sp.* could be used as antifungal (Table, 5). It has been reported that antifungal mechanism of antagonists has been attributed to the action of hydrolytic enzymes such as chitinase, β-1,3-glucanase, chitosanase, and protease (Wang *et al.,* 2002). The production of extracellular chitinolytic and β-1, 3-glucanolytic enzymes in the strain of *Streptomyces sp* were determined at different growth phases. The strain produced relatively high levels of chitinase (6.0 U/mg proteins) at day1 of the incubation period (Fig.1). Meanwhile, the highest level of β-1, 3-glucanase (0.82 U/mg proteins) was found at day 2 of the incubation period (Fig.2) and subsequently decreased slightly during the stationary phase (0.35.U/mg proteins). As stated in many previous reports (Kawachi *et al.,* 2001), the production of chitinase and β-1,3-glucanase enzymes by *Streptomyces* was related to fungal growth inhibition and the biological control of fungal pathogens due to the ability of *Streptomyces* to degrade fungal cell walls (Valois *et al.,* 1996; Mahadevan and Crawford ,1997 and Mukherjee and Sen ,2006)

Determination of minimum inhibitory concentrations (MIC) ranges of *C. albicans* and *Rhodotorula* sp isolated from clinical cases against tested antifungal compounds (Tables 6,7&8) showed that Streptomyces extract has antifungal activity significanly lower than Nystatin and near to ketoconazole and Itraconazole in most concentrations . It should be noted that one of the possible antifungal mechanisms of the *Streptomyces*

strain may be associated with the production of extracellular chitinase and β-1,3-glucanase enzymes. The finding that extracellular metabolites in the culture filtrates of the strain inhibited the growth of pathogenic fungi supported the investigation of the effects caused by its metabolites on the growth of the fungus. It has been reported that chitinase and β-1,3-glucanase enzymes are able to lyse fungal cell walls and are responsible for the suppression of fungal growth (Singh *et al.*, 1999). The antifungal agent of *S. violaceusniger* G10 showed *in vitro* antagonistic effects against *F. oxysporum* f.sp. *cubense*, such as hyphal swelling and the inhibition of spore germination (Getha and Vikineswary (2002). The fungal growth inhibition by the strain SAR14 is likely to be due to the presence of extracellular metabolites both hydrolytic enzymes and secondary antifungal compounds which could be used as alternative safe compounds for control of mycotic infections.

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